SYNTHESIS AND STRUCTURAL STUDIES OF CYCLIC Py-Im POLYAMIDES

Thesis by

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...for Kimberly, my love...

Acknowledgments

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Abstract

The work presented in this thesis is focused on the molecular recognition of DNA by minor groove binding polyamides. Methods and strategies for the solution-phase synthesis of hairpin and cyclic pyrrole-imidazole polyamides are presented with optimized protocols requiring little to no chromatography. These synthetic strategies have led to the design of cyclic polyamides targeted to the androgen response element and are shown to be biologically active and cell permeable in cell culture experiments in addition their binding affinities rival that of most polyamide architectures. The structural elucidation of an α-amino-turn-linked cyclic polyamide is presented at 1.17 Å resolution providing insight into the detailed molecular recognition process and allosteric modulation responsible for the inhibition of transcription factor-DNA binding. Additionally, structural elucidation of a β-amino-turn-linked cyclic polyamide, highlighting the conformational differences compared to the α-amino-turn linked structure is presented. A structural basis for the inability of polyamides to bind dsRNA is also proposed based on biophysical, structural, and modeling data. In addition to these studies a new class of programmable oligomers targeting the DNA sequence 5'-WGGGGW-3' were shown to inhibit DNA binding of the Nf-kB transcription factor by EMSA gel shift. Compounds synthesized in this study were found to possess unique fluorescent properties with the ability to modulate their fluorescence by binding their targeted dsDNA, leading to sequence specific fluorescent detection reagents. Efforts toward the templated-assembly of polyamides using higher-order DNA structure (NCP) are also reported and the development of a new profluorescent class of heterocycle, which has the potential to be used as a chemical reporter of ligation events is described.

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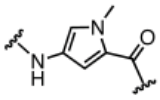
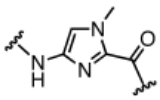
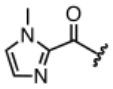
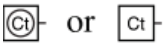
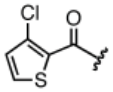
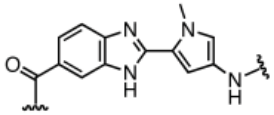
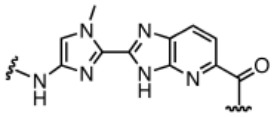
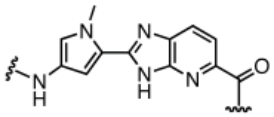
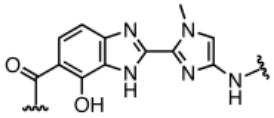
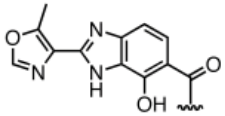
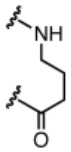
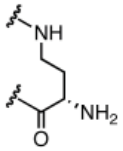
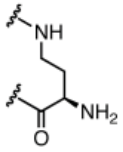
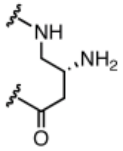
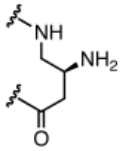
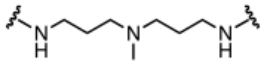
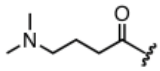
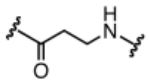
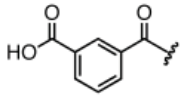
Nomenclature and Symbology

A	adenine
Å	angstrom
A•T	adenine Watson-Crick hydrogen bonded to thymine
Ac_2O	acetic anhydride
ADMET	absorption, distribution, metabolism, excretion, and toxicity
AP	activating protein
AR	androgen receptor
ARE	androgen response element
atm	atmosphere
Bi	benzimidazole
Boc	*tert*-butyloxycarbonyl
bp	base pair
ºC	degrees Celsius
C	cytosine
calc'd	calculated
Cbz	carbobenzyloxy
CCDC	Cambridge Crystallographic Data Centre
Ct	2-carboxy-3-chlorothiophene
Dbu	diazabicycloundecane
DCM	dichloromethane
DHT	dihydrotestosterone
DIEA	N,N-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
Dp	*N*,*N*-dimethylaminopropylamine
ds	double strand
Em	emission
ESI	electrospray ionization
Et	ethyl
Ex	excitation
FAB	fast-atom bombardment
Fmoc	fluorenylmethyloxycarbonyl
G	guanine
g	grams
G•C	guanine Watson-Crick hydrogen bonded to cytosine
GABA	gamma-aminobutyric acid
h	hour(s)
HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HF	hartree fock
Hp	3-hydroxypyrrole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry

hν	light
Hz	hydroxybenzimidazole
IC_{50}	median inhibition concentration (50%)
Im	*N*-methylimidazole
Ip	imidazopyridine
K_a	association constant
K_d	dissociation constant
λ	wavelength
m/*z*	mass to charge ratio
μ	micro
M	molar
max	maximum
MALDI	Matrix-assisted LASER desorption/ionization
min	minute(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry
N	normal
N	A, T, G, or C
NCP	nucleosome core particle
No	oxazole
NOESY	nuclear Overhauser enhancement spectroscopy
PCR	polymerase chain reaction
Py-Im	pyrrole-imidazole
PNA	peptide nucleic acid
PSA	prostate specific antigen
Py-Im	pyrrole-imidazole
RT-PCR	reverse transcriptase PCR
Py	*N*-methylpyrrole
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
OBt	hydroxytriazole ester
R_f	retention factor
RNA	ribonucleic acid
RP-HPLC	reverse-phase high performance liquid chromatography
sat.	saturated
satd.	saturated
ss	single strand
T	thymine
TFA	trifluoroacetic acid
TMR	tetramethyl rhodamine
TO	thiazole orange
TOF	time-of-flight
U	uracil
UV	ultraviolet
Vis	visible
W	adenine or thymine

	-Py-	
	-Im-	
	Im-	
	Ct-	
	-BiPy-	
	-ImIp-	
	-PyIp-	
	-HzIm-	
	NoHz-	

	-γ-	
	-*(S)*$^{\alpha\text{-}H2N}$γ-	
	-*(R)*$^{\alpha\text{-}H2N}$γ-	
	-*(R)*$^{\beta\text{-}H2N}$γ-	
	-*(S)*$^{\beta\text{-}H2N}$γ-	
	-(+)-	
	+)- or Dp-	
 -β-	-β-	
 or IPA–	-)- or IPA-	

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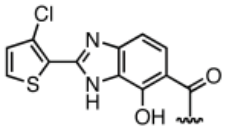

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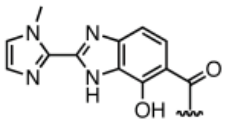

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